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Research Article

MOLECULAR PHYLOGENETIC ANALYSIS OF *ASOTA ORBONA* AND *ASOTA CARICAE* (LEPIDOPTERA: EREBIDAE) USING MITOCHONDRIAL COI GENE

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ABSTRACT

DNA barcoding has become the method of choice for a rapid, reliable, and cost-effective identification of pest species. Since DNA barcoding have proven to be highly efficient in identifying both immatures and adults, it is used to differentiate invasive and native pests. It has been used in managing species complexes in agricultural, IPM systems and also in the cases unpredictable species. Recently, DNA barcoding of partial mitochondrial COI gene is very popular in DNA based identification of various agricultural pest species. The present study investigates the molecular evolution of the *Asota* species using COI gene and its usefulness for reconstructing phylogenetic relationships within and among different *Asota* species.

Keywords: DNA barcoding, phylogenetic evolution, pest, mitochondrial COI gene.

INTRODUCTION

Asota is a genus of noctuoid moths in the Erebidae family (Lepidoptera: Insecta). Insect pests are one of the major concerns for farmers across the world and more than 10,000 species of insects have been recorded damaging the agricultural crops[1]. Detection of pests belonging to different groups is required for the protection of horticultural crops since they cause serious damage to various vegetation's.

In *Asota* species, palpi are upturned, where the second joint reaching vertex of head and third joint slender in variable lengths. In males the antennae is fasciculate but ciliated in females. Forewings with vein 5 is from the lower angle of cell or slightly just above from it. The 6th vein starts from the upper angle or below it. Areole is absent throughout in both. Hindwings have vein 5 from just above lower angle of cell. Veins 6 and 7 emerge from the upper angle.

However, keeping the shortcomings and limitations of the conventional taxonomical identification methods of identification of the pest species, DNA barcoding is used. A major feature of DNA barcoding is that it allows prompt identification of pest during young instars[2,3]. The mitochondrial DNA has been extensively analysed and proven to be an important tool in species delimitation as it possesses biological properties making it suitable as a marker for molecular biodiversity. Fragment size of mitochondrial cytochrome oxidase subunit I (COI) gene has been shown to provide high resolution to identify cryptic species, thereby increasing taxonomy-based biodiversity estimates and its usefulness has been confirmed for identifying Coleoptera[4], Diptera[5,6], Odonata[7], Hemiptera[8,9], Hymenoptera[10] and Lepidoptera[11,12]. DNA barcoding has proved to be a versatile tool with a variety of applications, for example, by facilitating the association between different developmental stages in insects.

MATERIALS AND METHODS

The experimental organisms, *Asota orbona* and *Asota caricae*, were collected from Kannur and Malappuram districts (Kerala: India) respectively. These are morphologically identified by expert consultation and preserved in 70% alcohol.

The genomic DNA in the homogenate was extracted using a GeNei Ultrapure Genomic DNA Prep Kit in accordance to the manufacturer's instructions. About 2 ng of genomic DNA was amplified for mitochondrial cytochrome oxidase subunit I (COI) gene using the specifically designed forward primer with nucleotide sequence 5'-GGTCAACAAATCATAAAGATATTGG-3' and reverse primer with sequence 5'-TAAACTTCAGGGTGACCAAAAAATCA-3'. The PCR reaction mixture consisted of 2ng of genomic DNA, 1µl each forward and reverse primers at a concentration of 2.5 µM, 2.5 µl of dNTPs (2mM), 2.5 µl of 10X reaction buffer, 1.20 µl of Taq polymerase (3U/µl) and 11.8 µl H_2O. The PCR profile consisted of an initial denaturation step of 2 minutes at 95 ^{0}C, followed by 30 cycles of 5s at 95 ^{0}C, 45s at 50 ^{0}C and 45s at 72 ^{0}C and ending with a final phase of 72 ^{0}C for 3 minutes. The PCR products were resolved on a 1% TAE-agarose gel, stained with Ethidium Bromide and photographed using a gel documentation system. After ascertaining the PCR amplification of the corresponding COI fragment, the remaining portion of the PCR products were column purified using Mo Bio Ultraclean PCR Clean-up Kit (Mo Bio Laboratories, Inc. California) as per the manufacturer's instructions. The purified PCR products were sequenced from both ends using the forward and reverse primers used for the PCR using Sanger's sequencing method[13]. The forward and reverse sequences obtained were trimmed for the primer sequences, assembled by using ClustalW and the consensus was taken for the analysis. The nucleotide sequence and peptide sequence were searched for its similarity using BLAST programme of NCBI (www.ncbi.nlm.nih.gov/) and Inter and intra specific genetic diversity were calculated using Kimura 2-parameter model with the pair wise deletion option and the difference in the nucleotide in codon usage partial COI sequence of *A. orbona* and *A. caricae* was analysed using MEGA6 software[14].

RESULTS AND DISCUSSION

The PCR of the COI gene fragment of *Asota orbona* (KX 603654) and *Asota caricae* (KU 201286) yielded product size of 525bp and 532 bp respectively. The BLAST search using the sequences revealed that the sequences obtained in this study was novel. The CO I gene in the mitochondrial genome has been proved to be an excellent source of information for the set of closely related families belonging to the order Lepidoptera. The evolutionary nucleotide divergence of *A. caricae* and *A. orbona* with various other *Asota* species is shown in Table 1.

Variation in the nucleotide is fundamental property of all living organisms which can be used for their identification and phylogenetic status. The average nucleotide frequencies for the species are A = 30.13%, T/U = 38.90%, C = 17.18% and G = 13.79%. The probability of substitution (r) from one base to another was calculated for 12 nucleotide sequences is shown in Table 2.

The evolutionary history was inferred using the Neighbor-joining method using COI partial sequence. The analysis of the evolutionary history of *A. orbona* and *A. caricae* was done using the Neighbor- joining method (Figure 1). Closely related species have 90% similarity in the standardized DNA sequence and distantly related species have less than 90% similarly in the same genus[15, 16].

Table 1: The evolutionary nucleotide divergence of *A. caricae* and *A. orbona* with various other *Asota* species

Organism with Accession No.	% of divergence
KX603654.1 *Asota orbona*	
KC499489.1 *Asota plana*	1.31%
HQ569654.1 *Asota paliura*	2.11%
HQ569644.1 *Asota heliconia clavata*	1.85%
KU201286.1 *Asota caricae*	2.68%
HQ569790.1 *Asota plaginota plaginota*	2.39%
KC499401.1 *Asota darsania*	2.39%
GU662416.1 *Asota albivena*	1.31%
HM395494.1 *Asota albiformis ternatensis*	2.11%
KF549916.1 *Asota eusemioides*	2.66%
GU662413.1 *Asota sulawesiensis*	2.40%
KJ013129.1 *Asota trinacria*	3.24%

Table 2: Maximum composite likelihood estimate of the pattern of nucleotide substitution. Each Entry shows the probability of substitution (r) from one base (Row) to another base (Column)

	A	T	C	G
A	-	2.0772	0.9176	5.2596
T	1.6092	-	22.2339	0.7364
C	1.6092	50.3319	-	0.7364
G	11.4940	2.0772	0.9176	-

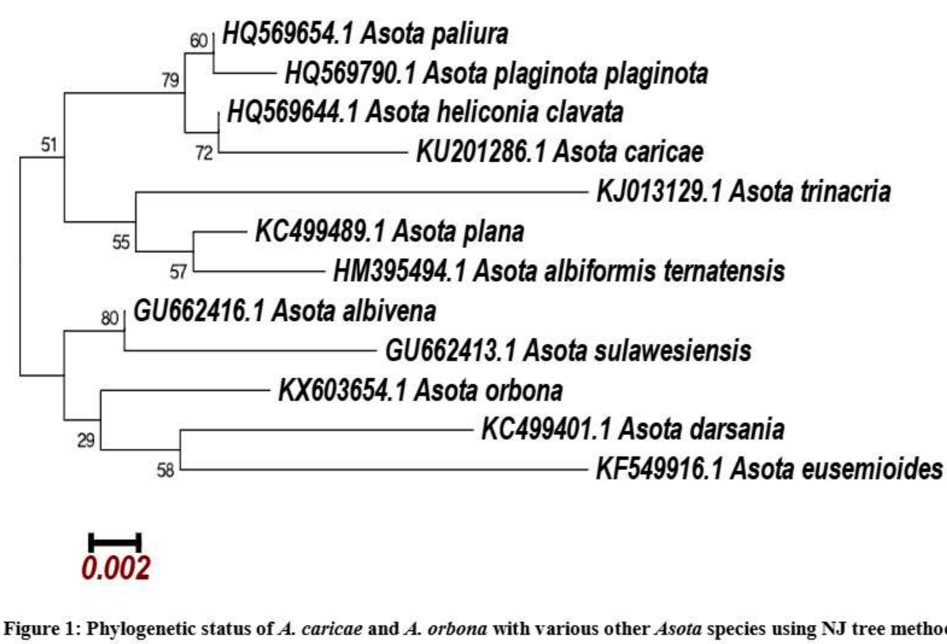

Figure 1: Phylogenetic status of *A. caricae* and *A. orbona* with various other *Asota* species using NJ tree method.

CONCLUSION

Variation within the genomic nucleotide sequence is one of the fundamental properties of all living organisms which can be used as the major criteria for unambiguous molecular level taxonomic identification and phylogenetic status analysis. The mitochondrial COI gene partial sequence obtained in this study showed nucleotide variation between the species *A. caricae* and *A. orbona* as 1.31%. Phylogeny analysis using NJ tree revealed the sharing of common ancestor for various *Asota* species and the two species *A. caricae* and *A. orbona*, is in a diverged clade. The phylogenetically close species of *A. caricae* and *A. orbona* are *A. trinaria* and *A. darsania* respectively. It is concluded that inter specific divergence of specific coding fragment of COI gene is very efficient for accurate species identification.

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